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EDITORIAL

On these pages the editor offers his opinions, unshackled by advertising patrons and unrestrained by anything save a sense of the decent and the truthful—the editor, alone, is responsible for their type, their tone and their tenor.

COMPLIMENTING CONNECTICUT

I HAVE before me as I write, Bulletin 415, issued by the Connecticut Agricultural Experiment Station. It contains in addition to other matter the thirtieth report on Drug Products. Because of this report I salute the pharmacists of Connecticut! By the same token compliments are due to pharmaceutical manufacturers the country over. Unquestionably, by the findings of this report, and by our common knowledge of things pharmaceutical drugs have been made safe for democracy.

Those who let the translucent membrane of history and hysteria obscure their view of the past are so prone to prattle over the "good old days." But in the field of medication the "good old days" were in some respects the *dumb* old days. The modern pharmacy, properly attended, dispenses and compounds remedial substances far more dependable, far more uniform, than ever did the old pharmacy, no matter how ethical was its conduct or up-to-date its technique.

The source of supply of medicinals has improved because of progress in the sciences supporting public health, and responsible pharmaceutical manufacturing houses are keeping faith with progress.

The ethics of pharmacy, fine as they are alleged to have been in the so-called "good old days" need be no less fine today.

Indeed the Connecticut report indicates that they are—substantial and worthwhile.

Here is clinical proof of improvement in pharmaceuticals. Here is definite evidence that in Connecticut, and more than likely in every state in the Union, modern pharmacy recognizes and fulfills its obligations in the faithful dispensing and compounding of medicinal agents and is accordingly accepting an opportunity to serve as well as a certainty to survive.

We have heard so much of the "open prescription counter." What we now more gladly hear is about the "open prescription conscience," that brooks no substitution, that realizes its obligations and fulfills them with exactitude.

And that alone is the measure of service that gives pharmacy its truly professional standing in the interest of public health.

From Bulletin 415 we quote:

Drugs

Blaud's Pills

Pills of ferrous carbonate (Blaud's pills) according to the U. S. P. specification contain in each pill not less than 0.06 gm. of ferrous carbonate, $FeCO_3$.

Twenty-three samples were examined for the Dairy and Food Commissioner.

None of the samples were substantially below standard, and as there is no upper limit officially fixed those showing large excesses are probably not in violation of the official standard. However, samples showing excess medicament greater than 10 per cent. are designated as too strong to distinguish them from samples that are closer to U. S. P. specification.

Syrup of Hydriodic Acid

According to the U. S. Pharmacopœia, syrup of hydriodic acid should contain not less than 1.3 nor more than 1.5 gms. of hydriotic acid per 100 cc.

Fifty official samples were examined and the analyses given.

Samples within the prescribed limits are regarded as satisfactory and those within 10 per cent. above or below are passed.

Thirty of the samples (60 per cent.) conformed strictly to the U. S. P. limits, and 10 of them (20 per cent.) were passed. Thus 80 per cent. were satisfactory or passable.

Syrup of Ferrous Iodide

Syrup of Ferrous Iodide according to the U. S. Pharmacopœia should contain not less than 6.5 nor more than 7.5 gms. of ferrous iodide in each 100 cc.

Of 78 official samples examined 31 were strictly within the official limits, and 45 were within 10 per cent. above or below those limits. All but two samples were either sastisfactory or passable.

Fowler's Solution

The U. S. P. standard for this preparation is not less than 0.95 and not more than 1.05 gm. arsenic trioxide (As_2O_3) per 100 cc.

Of 84 official samples 64 were within the U. S. P. limits and satisfactory, 19 were within 10 per cent. of those limits and passed, and one was below standard, though barely over the 10 per cent. tolerance.

Many of the samples were colored and flavored in accordance with the directions of the U. S. P. X but this practice is not in accordance with the present official text, U. S. P. XI.

And so might we continue, but sufficient evidence is already presented to justify rejoicing in the better state of pharmaceuticals, not just in the better State of Connecticut, but, by inference, the whole country over. But the report indicates that there is still a little room for improvement, in which there is continued challenge.

Ivor Griffith.

ORIGINAL ARTICLE

EXPERIMENTAL BRUCELLOSIS*

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IN a previous communication, the author, in conjunction with the Director of the Laboratories of Bacteriology and Hygiene of the Philadelphia College of Pharmacy and Science (1), presented a statistical survey of the prevalence and distribution of undulant fever and Bang's disease in the United States. As a result of that study, it appeared that factors other than those generally recognized might play a rôle in the transfer of brucella infections.

Purpose of the present research:

The present investigation was undertaken with the object of, first, to correlate and extent the work regarding parenteral infection with two species of brucella, namely *Br. abortus* and *Br. melitensis.* Some of this work has previously been done and reported by other investigators. However, in such cases, the experimental variations employed in this study, and the uniformity of dosage, adds confirmation to such findings, and expands our knowledge of brucella and their behavior in the living animal.

The second object was to make a simultaneous study of the two species, and establish their true relationship, insofar as the conditions of this experiment were concerned.

The third purpose of this study was to determine whether or not an arthropod vector might be capable of transmitting the infection among cattle, or from cattle to man. This purpose was brought to mind by careful statistical studies and observations which shall be dealt with in a separate communication.

General outline of research:

Parenteral infection was attempted by intravenous, subcutaneous, and intratesticular injections of a suspension of Br. abortus and Br.

*This paper covers part of a dissertation submitted to the Graduate School of the Philadelphia College of Pharmacy and Science in partial fulfilment of requirements for the degree of Doctor of Science.

melitensis, separately; by intravaginal application; by application of the suspensions to the unbroken and scarified skin; and by housing tested normal pigs with pigs giving positive blood agglutination tests. Later studies were made to determine the infectivitity of these organisms through copulation.

Oral administration was also tested for comparative results.

Finally, I attempted to determine the possibility of brucella infection being transferred by means of an arthropod vector. For this purpose, I selected ticks of the species *Dermacentor variabilis.* This species of tick is widely distributed over the eastern two-thirds of the United States (2), and is known to parasitize man, cattle and goats, all of which are susceptible hosts of brucella infection.

Cultures Employed:

These were obtained through the courtesy of Dr. I. Forest Huddleson of Michigan State College, and from the United States Public Health Service, and were as follows:

Culture No. 312—Br. melitensis, homo origin, isolated from the blood of an undulant fever patient in Tunisia, 1921.

Culture No. 382—Br. melitensis, homo origin, isolated from an undulant fever patient in Tunis, June 22, 1929.

Culture No. 56—Br. abortus, bovine origin, isolated April 17, 1928, in New York.

Culture No. 130—Br. abortus, bovine origin, isolated from a fetus in Maryland, September, 1917.

In later experiments, included in this report, Cultures No. 788 (Br. melitensis) and No. 1241 (Br. abortus) were also employed. These will be described under "Experiment 3—B."

Examination of the cultures, grown on liver infusion agar, showed them to be of the *smooth* or *S-types,* of characteristic microscopic appearance, which upon subculture revealed no change in type of colony, or in morphology.

Preparation of Antigen:

Antigens were first prepared separately from the four cultures previously described, according to the method described by Huddleson (3).

As each of these antigens showed such close uniformity in agglutinating power, a *polyvalent* antigen (prepared by combining equal amounts of each of the four antigens) was employed throughout this study.

EXPERIMENT 1

(1,500,000,000 organisms per cc.)

All guinea pigs used in this first experiment were maintained in the laboratory for two months prior to experimental use. During this time, a careful watch was maintained on their general physical condition, weight, behavior and general characteristics. Three blood agglutination tests made during this observation period were negative in all titers.

A forty-eight-hour culture of the organisms (S-type) was employed. The suspensions of the two strains of abortus (56 and 130) were combined; and in a separate sterile flask, the two strains of melitensis (312 and 382) were mixed. The concentration of the respective suspensions was adjusted to 1,500,000,000 organisms per cc., as determined by the McFarland nephelometer (4). In all cases, with the exception of skin applications, and intravaginal administration, 1.0 cc. doses were used.

Intravenous injections were made into the femoral vein. Subcutaneous injections were made in the median line of the abdominal wall. Testicular injections were made into the substance of the left testicle.

Intravaginal administrations were accomplished by inserting the end of a sterile glass syringe into the vaginal cavity, holding the animal up by the hind legs, and slowly introducing as much of the 1.0 cc. (under slight pressure) as could be accommodated. Under these conditions, saturation of the accessible reproductive system was assured.

Oral administration was easily accomplished by holding the pigs in an upright position, with the end of the sterile pipette placed in the side of the mouth, above the tongue. Following the introduction of each 0.25 cc., time was allowed for the animal to swallow the bacterial suspension. The procedure was repeated until the entire 1.0 cc. was consumed.

Application of the suspensions to the unbroken skin was made in the following manner. The hair was closely clipped from the major

portion of the back, the denuded area was washed with alcohol, dried, and to the area, so prepared, the 1.0 cc. of the bacterial suspension was applied in small fractional doses. After each application the suspension was *gently* spread over the surface of the prepared area with a cotton applicator, *care being taken not to rub in the suspension.*

Scarification of the skin was accomplished by scratching, with a sterile blunt needle, the denuded area on the back, prepared as described above. Care was taken to prevent free bleeding during the scarification. From here on, the procedure was carried out the same as described above.

The back of the animals was selected in order to *prevent licking of the treated area, and the subsequent ingestion of the bacterial suspension.* All pigs treated on the skin (unbroken or scarified) were isolated in individual cages, to prevent licking of the treated area by other animals similarly treated.

Contact infection was tested by placing tested normal guinea pigs, of the same sex, in the same cage with previously infected pigs, giving positive blood agglutination tests. All animals used in this test shared the same food and bedding.

Results of Experiment 1:

The results of blood agglutination tests made on the ninth day after the various treatments just described, are shown in Table 1. On the sixteenth day, all surviving animals (see column at extreme right of Table 1 for list of animals surviving for sixteen days or more), had developed agglutinins in titers up to 1:200 to 1:500.

Autopsies performed on all animals revealed no definite data. Although a number showed evidence of characteristic liver pathology, and the presence of hæmorrhagic and necrotic inguinal glands, I am uncertain as to the true interpretation of these findings without additional studies.

No significant blood or tissue cultures were obtained from animals employed in this experiment.

The early death of animals treated on the skin, and those under observation for contact infection (see Table 1), cannot be explained at this time.

The results obtained from the rapid test (Huddleson) (5), and the tube method were in close agreement (see Table 1).

TABLE 1

No.	SEX	METHOD OF ADMINISTRATION	BRUCELLA (Species)	9th DAY BLOOD AGGLUTINATION TESTS											DAYS FROM ADMINISTRATION OF ORGANISMS TO DEATH OF ANIMAL
				ANTIGEN CONTROL	RAPID TEST (Huddleson)					TUBE TEST					
					1:25	1:50	1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	
				+ Serum	+	+	+	+	+	+	+	+	+	+	
				− Serum	−	−	−	−	−	−	−	−	−	−	
1	M	Intravenous	Melitensis		+	+	+	+	+	+	+	+	+	+	12
2	M		"		+	+	+	+	+	+	+	+	+	−	13
3	F		"		+	+	+	+	+	+	+	+	+	−	9
4	M		Abortus		+	+	+	+	+	+	+	+	+	+	10
5	M		" *												6*
6	F		"		+	+	+	+	+	+	+	+	+	+	21
7	M	Subcutaneous	Melitensis		+	+	+	+	+	+	+	+	+	+	50
8	M		"		+	+	+	+	−	+	+	+	−	−	43
9	F		"												8
10	F		"												8
11	M		Abortus		+	+	+	+	+	+	+	+	+	+	9
12	M		"		+	+	+	+	+	+	+	+	+	+	25
13	F		"		+	+	+	+	+	+	+	+	+	+	38
14	F		"		+	+	+	+	+	+	+	+	+	+	15
15	F	Intravaginal	Melitensis		−	−	−	−	−	−	−	−	−	−	29
16	F		"		+	+	+	+	+	+	+	+	+	+	35
17	F		Abortus		+	+	−	−	−	+	+	−	−	−	16
18	F		"		−	−	−	−	−	+	+	−	−	−	10
19	M	Testicular	Melitensis												6
20	M		"		+	+	+	+	+	+	+	+	+	+	10
21	M		Abortus		+	+	+	+	+	+	+	+	+	+	10
22	M		"												8
23	M	Oral	Melitensis		−	−	−	−	−	−	−	−	−	−	51
24	M		"		−	−	−	−	−	−	−	−	−	−	9†
25	M		Abortus		−	−	−	−	−	−	−	−	−	−	49
26	M		"		−	−	−	−	−	−	−	−	−	−	12
27	M	Unbroken Skin	Melitensis		−	−	−	−	−	−	−	−	−	−	14†
28	M		Abortus		−	−	−	−	−	−	−	−	−	−	10
29	M	Scarified Skin	Melitensis												5
30	M		Abortus		+	+	−	−	−	+	+	−	−	−	13
31	M	Contact	Melitensis		−	−	−	−	−	−	−	−	−	−	13
32	M		"		−	−	−	−	−	−	−	−	−	−	8

* Positive in 1:25 and 1:50 on birthday, prior to death

† Accidental death

Discussion

Results of this experiment tend to indicate that with the dose and concentration employed, the formation of blood agglutinins appeared sooner following administration by the intravenous, subcutaneous, and testicular routes than by intravaginal or oral administration, by application to the unbroken or scarified skin, or by exposure to infected animals of the same sex. These findings are in agreement with those of Hagen (6), insofar as oral and contact infection is concerned.

No significant differences were observed in the formation of blood agglutinins, cultures, or autopsy findings which would suggest any variation between the species Br. *abortus and* Br. *melitensis.*

EXPERIMENT 2

(100,000,000 organisms per animal)

In this experiment, the concentration of the bacterial suspension and the dose were reduced; 0.2 cc. of a concentration of 500,000,000 organisms per cc. being given in all instances (actual dose, 100,000,000 organisms per animal).

In addition to the dose alteration, body weight and temperature studies were made, certain hematological studies were carried out, and additional methods of infection were attempted.

General routine of experiment:

All guinea pigs (thirty-six) used in this experiment were held under strict observation for two months. During this time the preliminary studies mentioned under the previous experiment were carried out, together with preliminary hematological, weight and temperature studies.

Preliminary agglutination tests:

Blood agglutination tests (rapid and tube) were made on the fifteenth, thirtieth, and fiftieth days of the observation period, were all negative in titers of 1:25, 1:50, 1:100, 1:200, 1:500.

Preliminary hematological studies:

At the time of bleeding the animals for the last preliminary agglutination tests (fiftieth day), leucocyte and differential counts were made. The smears were stained with Rapid Universal blood stain (Strumia).

Results of this study showed a fairly constant total leucocyte count; ranging from a minimum of 9000 leucocytes per cu. mm. to a maximum of 13,500 per cu. mm. The average was 13,000 white cells per cu. mm. of blood.

The following table shows the average differential count of normal guinea pig blood, obtained at approximately the same time (morning, before feeding) from animals maintained under uniform and controlled conditions.

TABLE 2

Average leucocyte count—13,000 per cu. mm.

Cells	Differential Count	Percent	Total Per Cu. mm.
Granulocytic	Rod Nuclears	0	0
	Neutrophilic Polymorphonuclears	35.5	4615
	Eosinophiles	0	0
	Basophiles	0	0
Lymphocytic	Prolymphocytes	0.8	104
	Lymphocytes	62.0	8060
	Leucocytoid Lymphocytes	0	0
Monocytic	Monoblasts	0	0
	Monocytes	1.7	221

This morphological blood examination shows considerable variation from that reported by Klieneberger and Carl (7), especially in the relative number of eosinophiles and lymphocytes. However, the total leucocyte count is in fair agreement with that reported by these workers (15,000 per cu. mm.).

Preliminary weight studies:

All guinea pigs were weighed immediately upon their arrival in the laboratory. At this time weights ranged from 295 to 515 grams. The average weight being 422 grams. On the fiftieth day the weights

ranged from 335 to 630 grams; average, 483 grams. During the two months' observation period the pigs employed in this experiment showed an average gain in body weight of 61 grams. All pigs, with the exception of two females, showed a satisfactory increase in body weight. (Note: The two females which had shown a decrease of 25 and 105 grams during the observation period, died three and seven days respectively, after being infected.)

Preliminary temperature determinations:

On the thirtieth and sixtieth days of the observation period the temperature of each animal was taken.

The temperatures varied from 99.0 degrees F. (37.2 degrees C.) to 102.0 degrees F. (39.0 degrees C.), giving an average of 100.5 degrees F. (38.0 degrees C.). The following tabulation shows the temperature range of all animals prior to infection with Br. abortus and Br. melitensis.

TABLE 3

Number of Guinea Pigs	Temperature Range
9	99.0°F. to 100.0°F.
19	100.1 101.0
8	101.1 102.0

Methods of administration of bacterial suspension:

As previously stated, the same methods of experimental treatment were employed as described under Experiment 1. The dosage employed in this experiment was, in all cases, 0.2 cc. of a 500,000,000 per cc. suspension, or 100,000,000 organisms per animal.

For determining the possibility of transmission of the infection (as determined by blood agglutination tests) through copulation, the following method was employed.

Two female guinea pigs were infected by means of intravaginal administration of the suspension; one receiving Br. abortus, the other Br. melitensis. Both of these pigs gave positive blood agglutination tests in titers of 1:500 at the time of mating with normal (negative

blood agglutination tests) male pigs. Each female was placed in a separate cage with the normal male.

Two male pigs were infected by means of testicular injection of the organisms; one with Br. abortus, the other with Br. melitensis. Both male pigs gave positive blood agglutination tests in titers of 1:500 at the time of mating. Each male pig was placed in a separate cage with a normal female with a previously determined negative blood agglutination test.

Similar studies were made with cultures of Br. abortus by Sanderson and Rettger (8). However, in their studies infection of the male was brought about by painting the penis on the inner surface of the urethra with suspensions of Br. abortus prior to mating with normal females. In their studies, these investigators produced infection in the female by painting the inner surface of the vagina with suspensions of Br. abortus.

It is impossible to state exactly when copulation occurred, but, in view of the fact that two of the three mated females (one having died five days after the mating) showed well advanced pregnancy when examined on the thirty-third day, one can definitely conclude that in these two cases copulation occurred shortly after having been mated.

Results on this phase of the experiment (Table 4) are reported in the number of days from the time the pigs were placed together in their respective cages. It may be noted at this point that the females referred to above delivered litters of two each on the sixty-eighth day (which is the accepted gestation period for the guinea pig). These young pigs appeared normal in their physical development, and gave negative blood agglutination tests on the tenth day after birth.

Nasal administration was accomplished by dropping into each nostril 0.1 cc. of the suspension. (There is little doubt but that some of this suspension eventually gained access to the gastro-intestinal tract.)

Results of Experiment 2:

The only initial reactions noted were among the animals which had received testicular injection; these, however, were much less severe than in the previous experiment. No perceptible difference in the degree of the reaction could be detected between that caused by Br. abortus and Br. melitensis. It was likewise only in this group

TABLE 4

No.	SEX	METHOD OF ADMINISTRATION	BRUCELLA SPECIES / Titer	10th DAY RAPID TEST 1:25	1:50	1:100	1:200	1:500	10th DAY TUBE TEST 1:25	1:50	1:100	1:200	1:500	15th DAY RAPID TEST 1:25	1:50	1:100	1:200	1:500	15th DAY TUBE TEST 1:25	1:50	1:100	1:200	1:500	20th DAY RAPID TEST 1:25	1:50	1:100	1:200	1:500	20th DAY TUBE TEST 1:25	1:50	1:100	1:200	1:500	REMARKS
		Antigen Controls	+ Serum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
			— Serum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
1	M		Melitensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
2	M		"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
3	M	Intravenous	Abortus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
4	M		"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5	M		Melitensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
6	M		"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
7	M	Subcutaneous	Abortus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
8	M		"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
9	M		Melitensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 63
10	M		"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
11	M	Testicular	Abortus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Accidental Dea 16th Da
12	M		"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
13	M		Melitensis	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
14	M	Unbroken	"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
15	M	Skin	Abortus	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
16	M		"	—	—	—	—	—	+	+	+	—	—	+	+	—	—	—	+	+	+	—	—	+	+	+	+	—	+	+	+	+	—	Died on 40
17	M		Melitensis	+	+	+	—	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 35
18	M		"	+	+	+	—	—	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
19	M	Scarified Skin	Abortus	+	+	+	—	—	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 61s
20	M		"	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
21	M		Melitensis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	Died on 61s
22	M		"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	+	+	+	+	—	
23	M	Oral	Abortus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
24	M		"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	+	+	+	+	—	Died on 30
25	F		Melitensis	+	+	+	—	—	+	+	+	—	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
26	F		"																														Died on 3rd
27	F	Intravaginal	Abortus	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
28	F		"																														Died on 7th
29	M		Melitensis	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
30	M		"	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
31	M	Nasal	Abortus	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 63r
32	M		"	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
33	M*		Melitensis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
34	F*		"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
35	M*	Copulation	Abortus																															Female Died 5 after Matin
36	F*		"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

M* Injected male mated with normal female.
F* Injected female mated with normal male.

ABLE 4

15th DAY								20th DAY										REMARKS
APID TEST			TUBE TEST					RAPID TEST					TUBE TEST					
1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 63rd Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Accidental Death on 16th Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
—	—	—	+	+	+	—	—	+	+	+	+	—	+	+	+	+	—	Died on 40th Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 35th Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 61st Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	Died on 61st Day
—	—	—	—	—	—	—	—	+	+	+	—	—	+	+	+	+	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	+	+	+	+	—	+	+	+	+	—	Died on 30th Day
+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
																		Died on 3rd Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
																		Died on 7th Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died on 63rd Day
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
																		Female Died 5 Days after Mating
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

that any significant alteration from the pre-infection temperatures was noted. Pigs which had received testicular injection showed an early temperature reaction, being 1.8 degrees F. above that of the average preliminary observation period.

Examination of all animals on the tenth day following administration of the suspensions, showed a marked local necrosis at the site of subcutaneous injection of Br. melitensis. Animals injected subcutaneously with Br. abortus showed only slight, or no necrosis. On this day, animals receiving intravaginal administration or testicular injection of Br. abortus, showed a more severe reaction than those treated in a similar manner with Br. melitensis.

Blood agglutination studies made on the tenth day were positive by both the rapid and tube methods, excepting those which had received oral administration of the bacterial suspensions, and those being used to test the infectivity of brucella through copulation (Table 4).

It is interesting to note that all animals in which nasal administration was employed had, on the tenth day, developed agglutinins in titers up to 1:200; while those to which a similar amount of the same suspension was administered orally, failed to show the presence of agglutinins in the blood on that day.

No significant weight or temperature alterations were noted after the fifth day, and it was not until the forty-fifth day following administration of the bacterial cultures, that any consistent change was observed in the blood picture. This finding, relative to weight alteration, is in agreement with that of Smith (9), and others.

On the forty-fifth day, a marked and consistent *leukopenia* was noted in both the Br. abortus and the Br. melitensis treated pigs, and both groups showed a *relative granulocytosis,* with an absolute reduction in the neutrophilic polymorphonuclears. Accompanying this change, a *relative and absolute lymphopenia in both groups was noted.*

No significant alteration in the blood picture was noted in the normal control pigs on this day, nor did the normal controls, on this day, show any significant alteration from the normal blood picture shown in Table 2. The blood picture at ten, twenty and forty-five days after experimental treatment, and for the normal controls, is shown in Table 5.

TABLE 5

Days from Inoculation	Leucocyte Counts Per Cu. mm.	Rods.		Polys.		Eosino.		Baso.		Prolymph.		Lympho.		Monos.	
		%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
NORMAL CONTROLS															
Preliminary (Normal)	13,000	0	0	35.5	4615	0	0	0	0	0.8	104	62.0	8060	1.7	221
10	14,170	1.0	142	40.5	5739	0.5	71	0	0	3.0	425	53.5	7581	1.5	212
20	13,140	0	0	35.5	4665	0	0	0	0	2.5	328	60.0	7884	2.0	263
45	11,900	0	0	29.0	3451	0	0	0	0	0	0	69.0	8211	2.0	238
BR. ABORTUS TREATED															
10	11,400	0	0	46.6	5313	0	0	0	0	1.3	148	49.8	5677	2.3	262
20	16,230	1.0	162	35.5	5761	0	0	0	0	2.5	406	59.0	9576	2.0	325
45	4,700	1.0	47	53.5	2515	0.5	23	0.5	23	1.0	47	41.0	1927	2.5	118
BR. MELITENSIS TREATED															
10	9,200	0	0	38.0	3496	0	0	0	0	0	0	57.0	5244	5.0	466
20	13,160	0	0	42.0	5527	0	0	0	0	5.0	658	49.0	6449	4.0	526
45	4,800	1.3	62	47.3	2271	0	0	0	0	3.7	178	45.4	2179	2.3	110

Discussion

The actual dose of organisms employed in this experiment was 100,000,000, regardless of body weight or method of administration.

With this dosage there appears to be very little difference in the time required to produce agglutinins in the blood, *regardless of the species of brucella, or the method of administration employed,* with the exception of oral administration and through copulation. When this number of organisms was administered orally, the time required

to produce blood agglutinins was, in general, twenty days; whereas, infection through copulation failed to produce positive blood agglutination tests even after a period of sixty days.

Local and general manifestations showed marked variations with the dose of organisms administered. In the first experiment, thirteen (40.6 per cent.) of the thirty-two animals used, died within the first ten days following the administration of the suspensions. In this experiment, no deaths occurred within the first twenty days, and only four deaths occurred within the first sixty days. These four animals showed, upon autopsy, no demonstrable evidence of brucella infection.

The data obtained from agglutination tests, weight and temperature observations, local and general reactions, and the blood pictures, suggests that the pathogenicity of Br. abortus and Br. melitensis is very closely related, if not identical.

As already stated, it was thought that brucella infection might be transferred through the medium of an arthropod vector, the most likely one to be considered being a member of the superfamily—*Ixodoidea.*

EXPERIMENT 3-A

Description of ticks:

Male and female ticks of the species *Dermacentor variabilis* were obtained through the courtesy of the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture. These were all young, active ticks when employed in this research.

Experimental primary hosts:

Two normal guinea pigs (negative blood agglutination tests) were injected subcutaneously with 1.0 cc. of a 150,000,000 mixed suspension of Br. abortus (No. 56 and 130—Huddleson, and 456 from the United States Public Health Service). Two other normal pigs were similarly injected with a mixed suspension of Br. melitensis (No. 382—Huddleson, and 428 from the United States Public Health Service).

Tick Feeding Device:

For restricting the ticks to the experimentally infected primary hosts, the following device, which is a modification or elaboration of that employed by the Bureau of Entomology and Plant Quarantine for this purpose, was constructed.

The apparatus (Fig. 1) is made of thin brass, and consists of a chamber (A) 15 mm. high and 17 mm. in diameter, around the top of which is a recess, or bezel, for supporting a plain or perforated

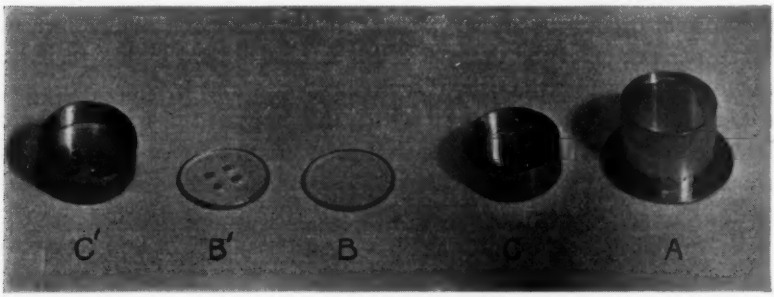

Fig. 1

glass window (B and B'). Around the entire base of this chamber is a flange, 5 mm. wide, by means of which the feeding chamber is attached to the back of the animal supported by a strip of adhesive about two inches wide, encircling the body of the animal, as shown in Fig. 2. After the desired number of ticks have been placed in the

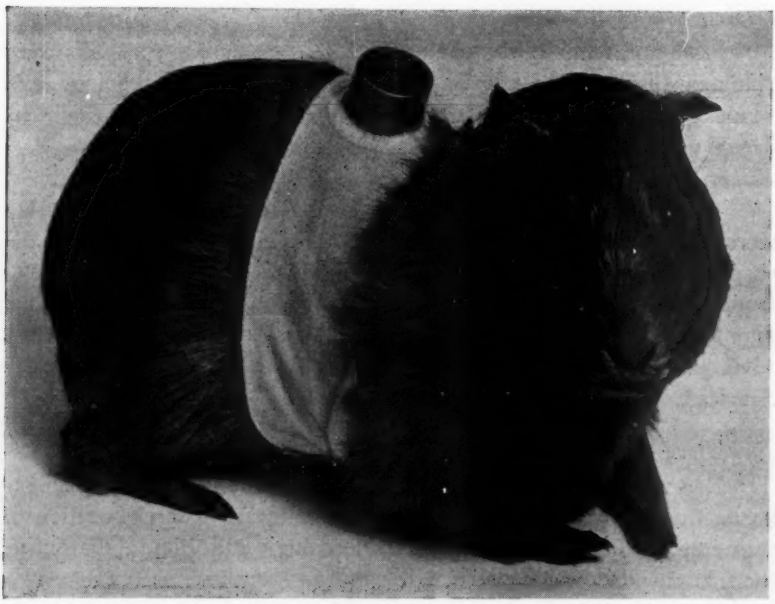

Fig. 2

chamber, and the glass window dropped into place, it is covered with a brass cap (plain or perforated) (C and C'). The cap fits over the chamber and is held in place by a ground joint, made tight enough to prevent it from being dislodged during the time when the ticks are on the animal. The glass window prevents the escape or crushing of the ticks when replacing the metal cap, and makes it possible for one to observe the ticks at all times. The plain window and solid metal cap are employed when feeding "seed" ticks (larvae) which otherwise could readily escape through the perforated top, used during the feeding of adult ticks.

This device has been employed most successfully throughout these experiments without the loss of, or injury to adults or larvae.

Feeding of ticks on infected hosts:

On the eleventh day following the subcutaneous injection of 150,000,000 organisms (abortus or melitensis), the blood of each pig was tested, and found positive in all titers up to 1:500. Four female and three male adult (normal) ticks were then placed on each infected animal, employing the above described device, which had previously been sterilized. On the fourth day, three of the four female ticks were found attached, and partially engorged with blood.

These ticks, together with the three males and one unengorged female, were removed from the pigs and immediately transferred to normal hosts.

Transfer of ticks to normal hosts:

The partially engorged ticks were placed into a sterilized feeding device, which had previously been attached to the back of normal pigs. The time consumed in making this transfer from the infected animals to the normal ones did not exceed twenty minutes. The normal pigs used in this experiment had been isolated for one month, during which time three negative blood agglutination tests had been obtained.

Results of Experiment 3-A:

All normal animals upon which *infected* * ticks fed, died in a period of time proportional to the time of tick feeding. Normal ticks fed on either normal or infected pigs, for periods of time exceeding that of the infected ticks, produced no demonstrable ill effects.

**Infected*—Ticks fed on infected guinea pigs.

Blood cultures and blood agglutination tests, made prior to the death of the animals reported above, were all negative.

Due to the unexplained death of the animals, the experiment was repeated, employing a new lot of ticks (same species), new strains of brucella, a smaller number of ticks per animal, and other slight technical alterations.

EXPERIMENT 3-B

Strains of brucella employed:

Strain No. 788—melitensis, and No. 1241—abortus, supplied by Dr. Huddleson, were employed.

Strain No. 788—Br. melitensis, isolated in December, 1935, from a case of undulant fever in the Argentine.

Strain No. 1241—Br. abortus, isolated from the milk of a cow, November, 1936.

Infection of primary hosts:

Infection of the primary hosts was obtained by injecting subcutaneously, 0.5 cc. of a 300,000,000 suspension of the above described organisms. Each suspension was injected separately into the animals serving as primary hosts.

Feeding of ticks on primary hosts:

Six days after the injection of the primary hosts, blood agglutination tests showed the presence of agglutinins in their bloods. Three female and two male adult ticks (normal) were then placed on each of the infected pigs. They attached themselves almost immediately.

Transfer of the ticks to normal hosts:

After ninety-six hours on the infected hosts, three female ticks were found well engorged with blood on both primary hosts. These, together with the two males, were removed and immediately transferred to normal guinea pigs, which had been isolated, and had given two negative blood agglutination tests. Here, as before, the tick feeding device had been sterilized prior to placing on the normal animals.

After seventy-two hours on the normal hosts, two of the three female ticks on each animal were fed to repletion. At this time all ticks were removed from the normal animals. The four fully en-

gorged females were then placed in pill boxes to oviposit. Oviposition started, in all cases, on the seventh day. The two remaining, partially engorged females were placed in tubes for further experimentation (to be presented in a later report).

Results of Experiment 3-B:

Blood agglutination tests run on the fifth, tenth and fifteenth days following the removal of the "infected" ticks, were all negative.

Further experimentation, in which the larvae from infected females were employed, and which has offered very interesting results, will be reported at a future time.

Summary

1—A comparison of the rapid (Huddleson) and the tube test, for the detection of brucella agglutinins, reveals a very close agreement between them. This observation is in agreement with those of Huddleson and Abell (10), and Welch and Mickle (11) (12), on human and cattle sera.

2—An average normal blood picture and temperature range for the guinea pig is presented.

3—A marked and consistent leukopenia was observed, following the administration of either Br. abortus or Br. melitensis. This was associated with a relative granulocytosis, and an absolute reduction in the neutrophilic polymorphonuclears. There was also noted a relative and absolute lymphopenia in the guinea pigs infected with either of the two species of brucella. These findings check closely with the more general ones reported by Gallagher (13), on the blood findings in human cases of undulant fever.

4—Guinea pigs infected by intravenous, subcutaneous and testicular injection, showed the presence of blood agglutinins (1:500) on the ninth day, regardless of the species or concentration of organisms employed in this study.

5—Animals treated on the unbroken and scarified skin with 100,000,000 organisms, revealed the presence of agglutinins in the blood sooner (with the exception of one treated on the unbroken skin with Br. arbortus) than animals treated in a similar manner with 1,500,000,000 organisms. However, the number of animals treated with the lower concentration was double that with the higher con-

centration. Therefore, further studies must be made to establish the validity of this observation.

6—Oral administration of Br. abortus and Br. melitensis failed to produce blood agglutinins as early as observed in the blood following infection by the methods mentioned under 4 and 5 of this summary.

7—Infection by contact, or through copulation, failed to produce blood agglutinins, detectable by either the rapid or tube methods. As stated elsewhere, this observation is in agreement with those of Hagen (6) and Sanderson and Rettger (8) with Br. abortus.

8—No consistent or significant weight alterations were noted after the fifth day following the administration of either of the two species of brucella. This observation is in agreement with that of Smith (9).

9—No consistent or significant temperature alterations were detected following the administration of either Br. abortus or Br. melitensis, except on the fifth day following testicular injection.

10—A simple and effective device for feeding ticks on experimental animals is described.

11—Preliminary studies on the possibility of ticks being a vector of brucella infections, failed to substantiate this idea, due to the early and unexplained death of animals upon which the infected ticks had fed. The results obtained in this phase of the work have proven of sufficient interest to encourage further studies along this line. Since the writing of the original thesis, further studies have been made, and will be presented in a later report.

12—In the original dissertation, an observation was reported dealing with the protective action of initial small doses of brucella to subsequent exposure to the large dose of these organisms, as employed in Experiment 1. In brief, it was noted that some protection was afforded pigs against doses of 1,500,000,000 organisms by first injecting 0.2 cc. of a 500,000,000 suspension of the organism, however, the results were not sufficiently conclusive to report in further detail at this time.

13—Certain serological observations were made as to the antigenic properties of monovalent and polyvalent brucella antigens.

Acknowledgments

I wish to express to all Federal and state agencies, and individuals of these agencies, who have so kindly cooperated in this study, and to Dr. Huddleson, my most sincere gratitude; and to Professor Gershenfeld, under whose direction this work was conducted, my most profound thanks.

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DIGITALIS DETERIORATION*

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THE subject of drug stability is obviously one of major importance to the pharmacist who seriously follows his chosen profession. At the present time it would appear that more attention is paid to the deterioration of biological preparations than of vegetable drugs. The pharmacist would not think of dispensing an old ampule of pituitary or an old sample of diphtheria antitoxin. Does he likewise consider deterioration when dispensing digitalis? I am afraid not. Nor is he necessarily to be condemned, for much of the question is in a state of unbelievable confusion. This is exemplified by the fact that after forty years of intensive investigation we do not seem to be able to answer in a definite fashion the elementary question, "Does tincture of digitalis deteriorate appreciably in six weeks, six months, or six years?" Let us review the problem and make such conclusions as might seem justified from the evidence at hand. The data presented represents the author's impression of the more recent literature pertinent to this question, and little in the nature of original research. Digitalis purpurea alone will be considered; other species and members of the "digitalis" family behave, generally, similarly.

Before entering upon a discussion of deterioration proper, it might be best to discuss first, the nature of the active principles of digitalis; second, the various methods for assaying digitalis, and third, the factors which seem to influence digitalis deterioration.

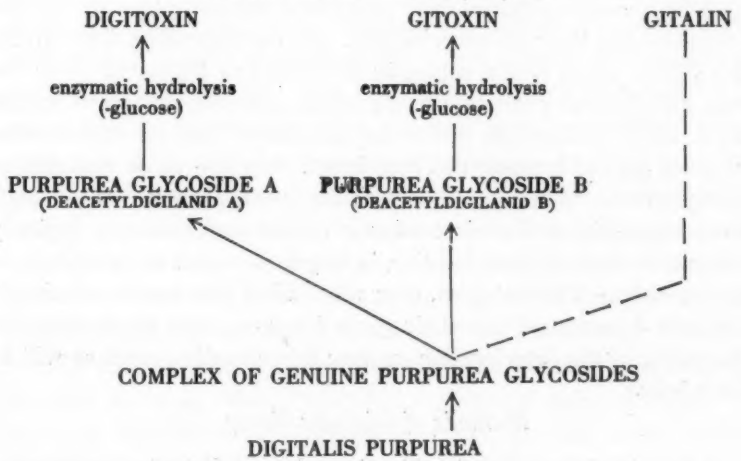
The Active Principles of Digitalis

One fact is definitely known about the active principles of digitalis, and that is, that they are glycosides. We might mention here that, generally speaking, glycosides are much less stable than alkaloids. From digitalis there have been separated three glycosides which apparently represent hydrolysis products from larger molecules, the cleavage occurring during the process of extraction. In other words, the glycosides, as we identify them after extraction, do not represent

the true state of the active principles in the intact leaf. Stoll called these larger non-split molecules the "genuine" digitalis glycosides (Fig. 1), and has devised a method whereby they might be extracted.

FIG 1. (From Stoll (1))

THE ACTIVE PRINCIPLE OF DIGITALIS



While not bearing directly upon the problem of deterioration, it might be pointed out here that if hydrolysis occurs during the preparing of tincture of digitalis from the powdered leaf, then the biological activity of the tincture might not necessarily represent the physiological potency of the whole leaf.

From digitalis purpurea there have been isolated three glycosides: digitoxin, gitoxin (bigitalin), and gitalin. (Fig. 2.) These are present

FIG. 2

THE GLYCOSIDES OF DIGITALIS PURPUREA

$$\underset{\text{Digitoxin}}{C_{41}H_{64}O_{13}} + 3H_2O = \underset{\text{Digitoxigenin}}{C_{23}H_{34}O_4} + 3\underset{\text{Digitoxose}}{C_6H_{12}O_4}$$

$$\underset{\text{Gitoxin (Bigitalin)}}{C_{41}H_{64}O_{14}} + 3H_2O = \underset{\text{Gitoxigenin}}{C_{23}H_{34}O_5} + 3\underset{\text{Digitoxose}}{C_6H_{12}O_4}$$

$$\underset{\text{Gitalin}}{C_{35}H_{56}O_{12}} + 2H_2O = \underset{\text{Gitoxigenin-hydrate}}{C_{23}H_{36}O_6} + 2\underset{\text{Digitoxose}}{C_6H_{12}O_4}$$

in the dry leaf to the extent of about 1 per cent. Chemically these glycosides are rather closely related to the sex hormones and to some of the carcinogenic substances.

These glycosides are characterized by the relative ease with which they are split into simpler compounds either by dilute alkalies, acids, or various ferments. In this connection it is interesting to note that vegetable ferments obtained from other than digitalis plant are capable of decomposing these active principles. On decomposition these glycosides yield a genin plus a sugar, as illustrated in Figure 2. It is the genin moiety that gives to the glycoside molecule the characteristic cardiac effect; though the genins are less active than the entire undecomposed glycoside upon the frog heart, they are more resistant to decomposition. When one considers the chemical nature of the digitalis constituents and their tendency to cleavage, one can logically assume that digitalis deterioration is largely a matter of hydrolysis of the glycosides. This being so, then when all of the "active principles" have been decomposed into their genin fractions, then there should be a flattening of the deterioration curves; this actually occurs as will be shown below.

Methods of Digitalis Assay

Of major importance in this discussion of digitalis deterioration is the question of the various methods which have been proposed for the study of digitalis potency. Several chemical methods have been suggested, none of which, however, seems to reflect consistently the physiological potency of the galenical preparation with satisfactory accuracy. This being so, reliance must be placed on various biological methods for calculating both the actual and relative toxicities of digitalis preparations. The two methods which have gained the most popularity are the official frog method and the cat method of Hatcher and Brody. The frog is a cold-blooded animal and in many respects much further removed physiologically from man than the cat. On the other hand, in the cat method, the question of absorption is not taken into consideration, since the drug is injected intravenously into an anesthetized animal, whereas, in the frog, it is injected into the anterior lymph sac from which absorption must take place before systemic effects can become manifested. This is a difference which may assume major importance; it is entirely possible to have a preparation shown to be quite active by the cat method and yet because of poor absorb-

ability show little activity by the frog method. Actually there does seem to be some evidence (2) that as tinctures of digitalis age, they become less absorbable. The limit of error allowed for both of these methods is rather large, the Pharmacopœia allowing plus and minus 20 per cent. on comparison of the unknown with the U. S. P. standard reference powder of digitalis. With the cat method usually the potency of the specimen under study is not compared with the standard but is expressed in definite absolute terms. That amount of digitalis which will kill 1 kgm. of cat, when slowly injected intravenously over a period of sixty to ninety minutes, has arbitrarily been selected as being 1 cat unit. The cat unit of a standard preparation has been accepted as 100 mgms. From this it is seen then that by the frog method relative toxicity is established, while by the cat method actual, approximately maximum, toxicity. Because of the abnormally great activity of the U. S. P. XI reference powder (3) (4) (5) when assayed on cats, it is probably fortunate that it is not used for comparison in carrying out the cat procedure; a preparation so standardized could easily be attacked as being "dangerous" from a clinical point of view. Certainly at present clinical dosage in terms of "cat units" is based entirely upon the conception of actual, rather than comparative, values.

Which of these methods is the better? This question has provoked an unusual amount of discussion, frequently acrimonious, and it is undoubtedly because of lack of agreement between the results obtained with these two methods that the present confusing state of affairs exists. Perhaps preparations had best be standardized by both methods; one for considering absorption and the other for considering absolute, more or less maximum, toxicity. Digitalis or digitaloid preparations intended for intravenous administration, such as strophanthin, should obviously be standardized by an intravenous technique. In addition to these two assay methods, there is a crying need for adequately controlled clinical observations to accompany these laboratory procedures, particularly when studying the question of the effect of aging. Fortunately this is now being undertaken by the Committee of Revision of the U. S. Pharmacopœia under the leadership of Dr. Henry Christian of Boston.

TABLE 1

EFFECT OF AGING ON DIGITALIS LEAF*

Age (Months)	Potency (Per cent drug strength) (Frog Method)
3	136
6	130
13	100
16	76
22	60
28	50

*Schmidt and Heyl, *A. J. P.*, *91*, 425 (1919).

TABLE 2

EFFECT OF AGING ON POWDERED DIGITALIS LEAF*

(Frog Method)

SAMPLE	PERCENTAGE LOSS				
	4 months	8 months	1 year	2 years	6 years
1	0	10	0	0	5
2	0	20	10	0	20
3	0	0	0	0	5
4	8	0	18	10	20
5	0	15	10	0	13
6	0	20	0	10	0
7	0	10	0	0	—
8	13	10	13	0	
9	—	—	—	—	5

*Rowe & Pfeifle, *J. A. Ph. A.*, *25*, 855 (1936).

Many factors have been mentioned as being concerned with digitalis deterioration. The presence of moisture is undoubtedly an important factor; above certain limits (the U. S. P. limits the water content of the dry leaf to 8 per cent.) the higher the moisture the greater the rate of deterioration. Of all the digitalis preparations the aqueous ones are the least stable. Light has been incriminated as being capable of hastening the decomposition of the active principles of digitalis and hence the almost universal custom of storing digitalis preparations in colored bottles. Polarized light has been specifically mentioned as a deterioration agent by Macht and Krantz (6), although this finding was not substantiated by other workers (7). Plant ferments undoubtedly play a part as has been demonstrated by several investigators. They seem to be inactivated by high concentrations of alcohol and other organic solvents. They are most active in an aqueous medium. As in chemical reactions in general, the higher the temperature the greater the rate of digitalis deterioration. Several studies have been reported on the effect of hydrogen-ion concentration on the stability of digitalis. Peculiarly enough acids tend to stabilize digitalis preparations, although acid menstrua are not particularly good solvents for the active principles. Alkalies likewise delay decomposition, but are less efficient than acids. (Table 4.) The presence of oxygen has been mentioned as a possible factor in hastening deterioriation, though it is probably of minor importance. Time obviously is a most important factor, the longer a digitalis preparation stands the greater will be its deterioration. Finally, the nature of the drug must be considered; some specimens seem to deteriorate much more readily than others and it has been noted that one active principle may deteriorate more quickly than another. Digitoxin is regarded as the most stable of the active principles.

Deterioration of Digitalis Leaf

Table 1, taken from a paper by Schmidt and Heyle, demonstrates their experience in studying the rate of deterioration of one sample of digitalis leaf. From their results it appears that, as judged by the frog method of assay, the dry leaf itself can lose about one-third of its activity in a year. More recent studies by Rowe and Pfeifle (Table

2) upon several specimens of dry digitalis leaf kept in open containers and assayed by the frog method show no appreciable loss over a period of six years. This is in keeping with the notation by Edmonds (8), who observed no change in the activity of the international powder when assayed after ten years by the frog method. Substantiating Rose and Pfeifle is also the observation of Gathercoal (9), who found samples of digitalis leaf of standard activity after twenty-five years as determined by the frog method. Haskell and Miller (10), also employing the frog method, found no change in the powdered leaf after one year. Haag and Hatcher (11), employing the cat method, found no change in a sample of digitalis that had stood for sixteen years. Gold and De Graff (12), likewise employing the cat method, found no change in several samples of digitalis leaf after two and one-half to five years. Apparently the greater evidence indicates that the powdered leaf does not deteriorate over a period of many years, either as determined by the frog method or by the cat method. It is fair to assume that tablets, capsules, and pills of digitalis leaf are just as stable.

Deterioration of Infusion

According to the observations of Pomeroy and Heyl (Table 3) infusion of digitalis, when assayed by the frog method, loses about one-third of its physiological activity in about three weeks. Similar

TABLE 3

EFFECT OF AGING ON INFUSION OF DIGITALIS

(Frog Method)*

Initial Activity	90% U. S. P.
10 days	75% U. S. P.
22 days	60% U. S. P.
39 days	35% U. S. P.

*Pomeroy & Heyl, *A. J. P.*, *92*, 394 (1920).

results are reported by Hintzelman and Joachimoglu (13). On the other hand, employing the cat method, Hatcher and Eggleston (14), found no change in an infusion, frequently exposed to the air, after twenty-one days and even after two and one-half years. After eleven years a 50 per cent. loss of activity was noted. Haag and Hatcher (11), again employing the cat method, reported one infusion as having lost 75 per cent. of its activity after eight years, though it was still effective in appropriate dosage when employed clinically.

Deterioration of Tincture of Digitalis

Emig studied, by the frog method, the effect of aging on tincture of digitalis buffered with acid and also buffered with alkali. His results are given in Table 4 and show the remarkable deterioration of about 80 per cent. in a period of one year in the case of the control tincture. Tinctures buffered with acid or alkali were much more stable. Tables 5 and 6, illustrating the work of Wokes (15), (16), show a less rapid rate of deterioration, and is more or less in keeping with the observations made by other investigators when studying the problem of the deterioration of tincture of digitalis by the frog method.

TABLE 4

EFFECT OF AGING (1 YEAR ON POTENCY OF TR. DIGITALIS (Frog Method)*

Preparation	Percentage Activity Lost		
	Control	Buffered with Acid	Buffered with Alkali
Sealed	83	11.2	44
Unsealed	83	22	30
CO_2	67	22	30
In Refrigerator	83	22	20

*Emig, *J. A. Ph. A.*, *21*, 1273 (1932).

TABLE 5

EFFECT OF AGING ON TINCTURES OF DIGITALIS

(Frog Method)*

Tincture	Age of Tincture						
	Three Months	Four-and-Half Months	Six Months	Seven-and-half Months	Nine Months	Fourteen Months	Sixteen Seventeen Months
1	94	—	75	—	66	70	60
2	—	—	77	—	—	66	—
3	93	—	81	—	63	—	—
4	105	—	97	—	77	—	—
5	80	—	82	—	71	—	—
6	—	81	—	76	—	—	—
7	86	—	78	—	84	—	—
8	85	—	97	—	76	—	—
9	—	87	—	87	—	—	—
10	—	88	—	82	—	—	—
11	—	90	—	79	—	—	—
12	—	84	—	85	—	—	—
12	—	—	—	—	—	64	—
Average	91	86	84	82	73	67	60

*Wokes, *Quart. J. Pharmacy and Pharmacol.*, *2*, 48 (1929).

TABLE 6

RELATION BETWEEN INITIAL POTENCY AND RATE OF DETERIORATION OF DIFFERENT TINCTURES OF DIGITALIS, AS SHOWN BY THE FROG METHOD*

Tincture	Initial Potency (as per cent. of that in Tincture Freshly Prepared from International Standard Powder)	Potency at end of Nine Months	Loss of Activity during, Nine Months' Storage (as per cent. of Initial Potency)
1	150	95	37
2	134	95	29
3	102	79	23
4	99	83	16
5	85	65	24

*Wokes, *Quart. J. Pharm. and Pharmacol.*, *3*, 205 (1930).

Incidentally, Figure 3 by Wokes (16) illustrates the leveling-off process of deterioration mentioned previously. Gunn (17) studied the rate of deterioration of tincture of digitalis on frogs and found no loss after 1 year, 10 to 15 per cent. after two years, and 20 per cent. after eleven years. Munch (18), in his collaborative research on the deterioration of tincture of digitalis employing the official method of assay and sponsored by the American Pharmaceutical Association, reports distinct loss in activity for the first several years and then gradually an *increase* in activity. Table 7 from a paper by Haag and Hatcher, shows the effect of aging on the potency of tincture of digitalis when standardized by the cat method. It is interesting to note that tincture 1 of this series lost 50 per cent. of its potency in six months but showed no further loss after nine years. Their table illustrates rather strikingly (a) the great difference in the rate of deterioration of various

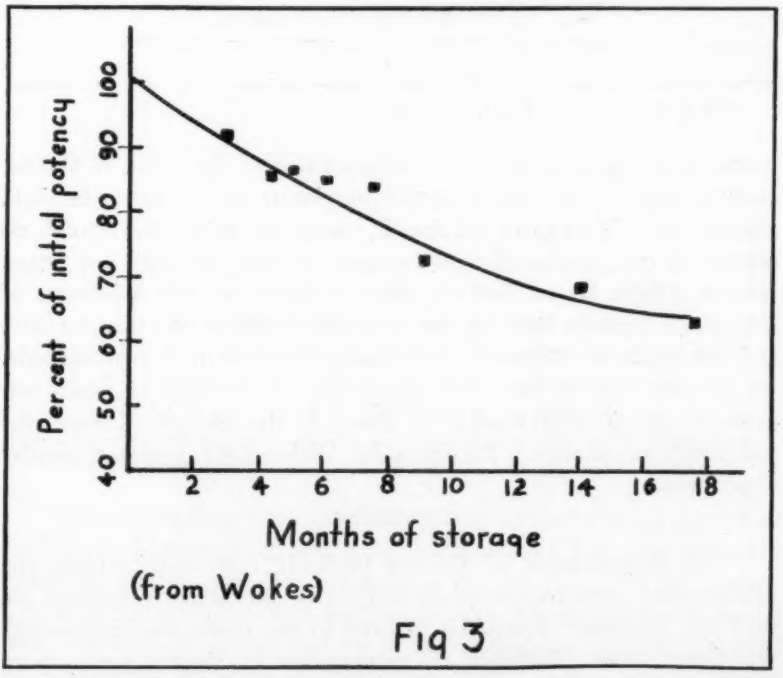


FIG. 3. Loss of activity of tincture of digitalis as shown by the frog method. Ordinates represent percentage of initial potency; abscissae represent time of storage (at room temperature) in weeks. The curve is a composite one representing the average loss of activity in thirteen different tinctures, each of which was assayed at intervals against a tincture freshly prepared from the same batch of leaves.

TABLE 7

EFFECT OF AGING ON POTENCY OF TR. DIGITALIS
(Cat Method)*

Tincture	Age (Years)	Percentage Activity Lost
1	½ 9	50 50
2	5	14
3	6	50
4	7	14
5	11	35
6	16	38
7	16	27
8	16	50
9	18	25

*H. & H., *J. A. M. A.*, *93*, 26 (1929).

tinctures of digitalis, and (b) that according to the result of the cat assay procedure, tinctures generally deteriorate quite slowly. Haskell, Daniel, and Terry (19) (Table 8) assaying with cats, found no change in the activity of four tinctures of digitalis after five years. Stasiak (Table 9) studied the effect of aging on five specimens of tincture of digitalis both by the cat method and by the frog method, and has nicely illustrated the discrepancy between these two methods. By the frog method there was appreciable (and if real, clinically important) deterioration after three years; by the cat method there was practically no change. Figure 4 by Wokes (16) shows a similar experience.

Conclusions

The deterioration of digitalis probably rests chiefly upon the fundamental phenomenon of hydrolysis of the active principles, be they the "genuine" glycosides of Stoll or the more simple generally recognized ones. Evidence of detrioration is shown much more definitely by the frog method than by the cat method. It should be established once and for all and to the satisfaction of all concerned, if possible, which of these two methods more clearly reflects the clinical efficiency of digitalis, particularly on aging.

TABLE 8

SUMMARY OF RESULTS OF CAT ASSAYS IN 1917 AND IN 1922 OF FOUR TINCTURES OF DIGITALIS*

No. Sample	1st Assay mg/Kgm.	Time Lapse in Months	2nd Assay mg/Kgm.	% Change
1	55.7	64	62.5	—12.2
2	68.2	61	70.0	— 2.6
3	42.2	63	40.8	+ 3.3
4	71.2	64	64.0	+11.2

*Haskell, Daniel and Terry, *J. A. Ph. A.*, *11*, 918 (1922).

TABLE 9

EFFECT OF AGING (3 YEARS) ON TR. DIGITALIS (Frog and Cat Method)*

Preparation	Percentage Lost	
	Frog Method	Cat Method
1	25	2.2
2	38.5	16.8
3	58.2	6.5
4	58.2	10.3
5	60.0	20.6

*Stasiak, *Arch. Pharm.*, *272*, 743 (1934).

Both by the frog method and by the cat method the dry powdered digitalis leaf seems to be stable for many years. The same probably holds for tablets, capsules, and pills of the dry leaf. To be entirely safe and free from criticism digitalis infusion should not be dispensed after one week of storage and the supply of tincture of digitalis should be replenished every six months to one year. While little is known about the rate of deterioration of liquid proprietary preparations of digitalis, it is known that they deteriorate and it is best that they too not be dispensed after one year.

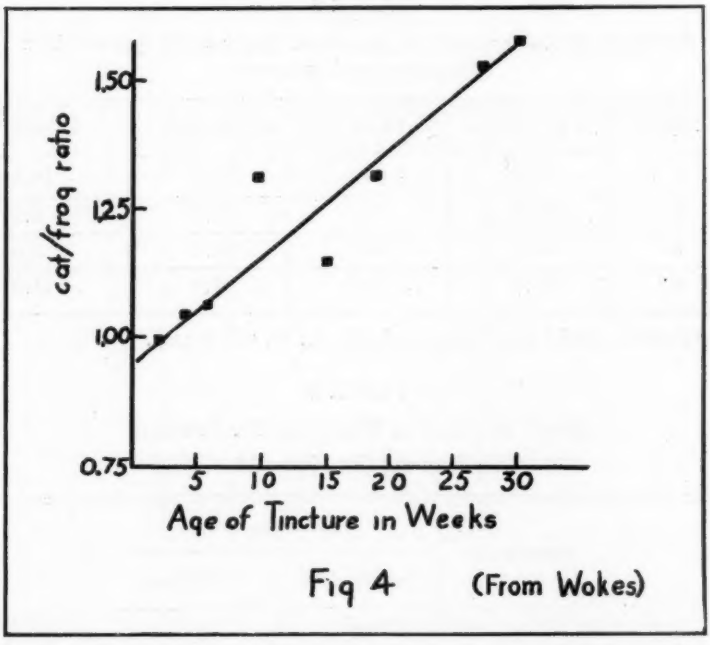


FIG. 4. Showing increase in cat/frog ratio, due to decrease in frog potency, during aging of tincture of digitalis. Ordinates are cat/frog ratios, abscissae age of tincture in weeks.

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ABSTRACTS FROM AND REVIEWS OF THE LITERATURE OF THE SCIENCES SUPPORTING PUBLIC HEALTH

Bacteriology . . . . Louis Gershenfeld, B. Sc., Ph M.
Biochemistry, Nutrition, etc. . . . Arno Viehoever, Ph. D.
Biology . . . . . Marin S. Dunn, Ph. D.
Chemistry . . . . . Arthur Osol, Ph. D.
Pharmacy . . . . . E. Fullerton Cook, Ph. M.
and their assistants

The Prevalence of Trichinosis and Measures for Control. M. C. Hall. *Public Health Reports 53,* 1472 (1938). In a previous abstract (*Amer. J. Pharm. 110,* 200 (1938)) attention was directed to a paper which reported upon the high incidence of trichinosis in this country. The present paper summarizes the findings of the Public Health Service as reported in a series of related papers on this subject. They are as follows:

1. Abundant evidence indicates quite clearly that trichinosis is a greater problem in United States than in any other country.

2. Human trichinosis rests primarily on swine trichinosis.

3. Swine trichinosis rests primarily on the practice of feeding uncooked or inadequately cooked pork scraps to swine as garbage, table scraps, etc.

4. The indicated control of trichinosis in the United States is largely a matter of keeping such uncooked pork out of the feed of swine and either cooking such feed as garbage (which in some countries is a legal requirement) or refraining from feeding any food containing pork scraps.

5. Rats appear to have a very minor role in the production of porcine trichinosis and may be given only secondary consideration in control measures.

6. The evidence available indicates that control measures employed in the past fifty years have not lowered the incidence of the infestation.

7. Prompt and effective control measures must be instituted at once if the swine industry and the packing industry are to be saved from extensive losses as a result of public opinion.

A plan for effective control is outlined involving a program of co-operative effort on the part of all groups concerned in this question. L. F. T.

Synthetic Silk. Anon. *Science Supplement 88,* 8, September 30, 1938. United States Patent No. 2,130,948 issued to the late W. H. Carothers, chemist of the E. I. du Pont de Nemours Company, describes the production of a new artificial silk from long chain amine compounds. These are prepared by reacting diamines and dibasic acids yielding acid salts which are crystalline in nature and have fairly definite melting points. Eight specific ways of creating the new fibers are described, a typical reaction mixture being 14.8 parts of penta-methylene-amine, 29.3 parts of sebasic acid and 44 parts of mixed xylenols.

These new completely synthetic fibers are easily drawn out to one-tenth the diameter of a natural silk filament and under extreme conditions may be reduced to one-seventy-fifth the diameter of the natural product. The new fiber possesses a tensile strength equal to or better than that of silk. Lustrous and silky in appearance, the fibers are almost completely insensitive to moisture. It is stated that when made into fabrics the synthetic fiber fabric possesses a far better elastic recovery than natural silk. A. O.

Note on the Extraction Method for Santonin in Mixtures. I. S. Shupe. *J. Assoc. Off. Agr. Chem. 21,* 515 (1938). The following method has been found convenient for the direct gravimetric determination of santonin in liquid preparations containing emodin and other plant extractives as well as in other mixtures. Transfer the accurately measured or weighed portion of sample to a suitable separator, make slightly acid with hydrochloric acid, and extract with portions of chloroform to remove all the santonin. If necessary, evaporate the chloroform to about 100 cc. and shake thoroughly with 20 cc. of 10 per cent. sodium hydroxide solution in a separator. Draw off the chloroform and wash with 20 cc. of water. Filter the chloroform through cotton or filter paper into a tared beaker and use 25 cc. of chloroform to wash the sodium hydroxide solution and

water, the chloroform portions then being combined. Evaporate the chloroform, dry at 100 degrees C. for thirty minutes and weigh the residue. Determine the melting point of the residue to ascertain whether it is pure santonin. Further purification of the santonin may be effected by treating it with barium hydroxide solution, filtering and re-extracting the santonin after acidification. Neutral substances or oils can be removed by extracting the alkaline solution of santonin with chloroform before acidification.

In the above method advantage is taken of the presence of the rather stable lactone group in santonin to separate it from most of the other acidic, resinous, phenolic, or alkaloidal compounds. In chloroform solution santonin is not affected by 10 per cent. sodium hydroxide solution. In the dry state it reacts with alkali to form water-soluble salts which yield santonin upon acidification. It is noted that chloroform will extract santonin quantitatively from acidified solutions containing as much as 50 per cent. of glycerol.

A. O.

An Evaluation of Cold Vaccines Based on a Controlled Study. H. S. Diehl, A. B. Baker and D. W. Cowan. *J. A. M. A. 111,* 1168 (1938). A carefully controlled study of the value of three different vaccines which were recommended for the prevention of colds was conducted on a large group of cold-susceptible students of the University of Minnesota.

A control group was observed during each year of the study. Such groups were chosen at random from students who applied for cold prevention treatment; the members were treated in exactly the same manner as those of the vaccinated group and they believed throughout the period of the experiment that they were receiving vaccine. In such controls sterile physiological salt solution was administered hypodermically in place of the vaccine or lactose filled capsules in place of the vaccine orally administered.

One of the most significant aspects of the study was the great reduction in the number of colds which the members of the control groups reported during the experimental period as compared to the number that the same students reported for the previous year. In fact, these results were as good as many of those reported in uncontrolled studies which recommend the use of cold vaccines. The group which received vaccine subcutaneously experienced an average of 25

per cent. less colds per person than did the control group. Although this difference is statistically significant it does not seem of practical importance since a reduction of 25 per cent. in the average number of colds in a group of individuals is not sufficiently great to justify the time and expense involved in carrying out the intensive vaccination procedure which was utilized.

The group which received the polyvalent oral vaccine experienced just as many colds as the control group during both years of study.

Although not entirely conclusive, there was no evidence found in this study either that vaccines reduce the complications of colds or that the condition of the nose and throat is related to the frequency of colds in a cold-susceptible group. L. F. T.

The Photometric Determination of Cystine, Cysteine, Ascorbic Acid, and Related Compounds with Phospho-tungstic Acid. B. Kassell and E. Brand. *J. Biolog. Chem. 125,* 113-129 (1938). A method is described for the determination of cystine and cysteine in which the Folin-Lugg procedure has been modified; this photometric method furnishes accurate results in the presence of other reducing agents. In addition, the method has been extended to a micro-modification for the estimation of these substances. A photometric procedure for the determination of ascorbic acid is described, as well as a method which permits the simultaneous determination of cystine, cysteine, and ascorbic acid. Results are tabulated from a study of comparative methods. A discussion of spectrophotometric colorimetry is included. L. A. R.

The Colorimetric Determination of Sodium as Uranyl Manganese Sodium Acetate. W. C. Woelfel. *J. Biolog. Chem. 125,* 219-227 (1938). A method is described whereby small amounts (as little as 0.1 milligram) of sodium may be determined in urine or blood serum. The sodium is precipitated quantitatively as uranyl manganese sodium acetate. This precipitate is dissolved, its manganese oxidized by potassium periodate to permanganate; the latter is then estimated by colorimetric comparison with suitable standards. L. A. R.

The Determination of Alcohol in Pharmaceutical Liquids. K. Bambach. *J. Ind. & Eng. Chem. Anal. Ed. 10,* 541 (1938). The author recommends the use of the chain hydrometer in the determina-

tion of alcohol in pharmaceutical products. This instrument was invented and developed by C. W. Foulk of Ohio State University and its construction and use is described by him in *J. Optical Soc. Am. 7*, 327 (1923).

The use of the chain hydrometer has been found to save considerable time in the determination of alcohol without sacrifice of accuracy. Alcohol-water temperature charts are described and illustrated by means of which chain hydrometer readings or pycnometer weights at room temperature can be converted directly to alcohol percentages at the official temperature. L. F. T.

Final Report on Sulfanilamide Elixir Poisoning. A Clinical and Experimental Correlation. E. Geiling and P. Cannon. *J. A. M. A. 111*, 919 (1938). As the result of extensive animal experimentation the following conclusions seem warranted.

1. Diethylene glycol was the chief toxic component of the Elixir of Sulfanilamide-Massengill examined, because experimental animals given diethylene glycol alone exhibited essentially the same clinical course and pathologic changes in the kidney and liver as did those treated with similar doses of Elixir of Sulfanilamide-Massengill or a "synthetic" elixir containing the ingredients in the same proportion as found by analysis in the Massengill preparation.

2. Sulfanilamide alone, if given in doses of 0.2 gm. per kilogram three times a day, did not prove fatal to rats, rabbits, after eight or more divided doses. However, when sulfanilamide was given in this dosage in the form of Elixir of Sulfanilamide-Massengill or in the "synthetic" elixir, either preparation was fatal and the experimental animals presented a clinical and pathologic picture closely resembling that reported for the human cases in which death occurred.

3. Although sulfanilamide alone did not produce the renal and hepatic changes caused by the elixir, one must not overlook the possible damage to tissues that may result when sulfanilamide is administered to experimental animals or to human beings with impaired renal function.

4. The pathologic picture was essentially similar in the three species of animals whether given the Elixir of Sulfanilamide-Massengill, the "synthetic" elixir or the diethylene glycol alone.

A comparison of the clinical picture in human cases of poisoning is made with those obtained in animal experimentation. L. F. T.

RECENT INVESTIGATIONS ON THE TUBERCLE BACILLUS AND ITS PREPARATIONS

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Philadelphia College of Pharmacy and Science

Numerous investigations and valuable researches on the tubercle bacillus, its derivatives and preparations were presented during the past year by many workers. Only the most interesting presentations are given.

Chemical Studies of the Dissociates of the H-37 Human Tubercle Bacillus. Gustave Martin. *J. Am. Chem. Soc. 60,* 768 (1938). Two variants of the human (H-37) tubercle bacillus, designated as Rv (virulent) and Ra (avirulent), isolated several years ago have maintained their biological and morphological characteristics. However, great differences were found in the total amount of lipoid extractable from the two variants. 24.5 per cent. of the Rv and 16.6 per cent. of the Ra organism were extractable by the use of simple organic solvents. The lipoid was extracted readily from the Rv variant but difficulties were encountered when extracting the Ra variant. A large amount of phosphatide appeared in the first extract from the Ra variant but not in the first extract from the Rv variant.

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The Influence of Hemoglobin and Ferrous Ammonium Sulfate on the Growth of the Tubercle Bacillus. R. Davies. *J. Path. Bact. 45,* 773 (1937). Ferrous ammonium sulfate and hemoglobin have been found to possess an inhibitory action on the growth of a virulent human type tubercle bacillus in an egg medium.

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On the Effects in the Rabbit of the Intravenous Injection of Massive Doses of Human Tubercle Bacilli. J. Valtis and F. Van Deinse. *Compt. rend. soc. biol. 126,* 495 (1937). The intravenous injection into rabbits of large doses (5-15 mg.) of human tubercle bacilli produced, in a large percentage of cases, a rapidly fatal infection which on autopsy resembled the type of infection produced by bovine bacilli.

Atmosphere Pollution in Relation to Tuberculosis. Pendrill Varrier-Jones. *Diseases of the Chest 4,* 8 (1938). The hypothesis is presented that zinc protects against tuberculosis but favors cancer development through stimulating the formation of tuberculosis bacteriophages. The latter destroy the tubercle bacilli but later may give rise to cancer. The zinc content of smoke might be responsible for its alleged tuberculosis protective carcinogenic action.

. . .

Concentration of Tubercle Bacilli From Sputum by Chemical Flocculation Methods. John H. Hanks, Harold F. Clark and Harry Feldman. *J. Lab. Clin. Med. 23,* 736 (1938). The addition of alum in the sodium hydroxide solution used for digesting sputum samples is recommended. Upon neutralizing the sputum digest the alum flocculates and carries down with it the tubercle bacilli.

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Bactericidal Action of Various Substances on Tubercle Bacilli. E. Baumann. *Klin. Wochschr. 17,* 382 (1938). Tubercle bacilli in vitro were killed by 1 per cent. sodium, potassium and ammonium sulfocyanate in acid solution in ten minutes, and by 0.5, 1 and 5 per cent. pyridine in 30 per cent. alcohol, in two hours, one hour and ten minutes, respectively. No bactericidal action was given by the thiocyanates in neutral or alkaline solution or by pyridine in water. The growth of tubercle bacilli in vitro was inhibited by biacetyl after two hours and by acid gastric juice after twenty-four hours. No effect on the growth of tubercle bacilli was given in vitro by 30 per cent. alcohol euflavin, Trypaflavin, Rivanol, Prontosil, Congo red, indigocarmine, Pyridium or thiazine. The tubercle bacilli were not affected by sulfanilamide, sulfanilic acid, formic acid, sodium formate, lecithin, resorcinol, Yatren 105, a bile acid compound, colloidal silver solutions, quinine hydrochloride, salicylic acid, sulfuric acid, sodium benzoate, diacetyltannin and vitamins A, B, C and D. The reactions of other substances were also presented.

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Merthiolate in Treatment of Pulmonary Tuberculosis. S. M. K. Mallick, Ali. Shujjat and Balbir Shingh. *Tubercle 19,* 62 (1937).

The use of Merthiolate in twelve cases of pulmonary tuberculosis given intravenously in 5 cc. doses (1:1000 aqueous solution) proved uneventful. No improvement was noted at the end of the treatment. Tubercle bacilli were found in the sputum in spite of the injections. In fact the number actually increased in 50 per cent. of the patients during treatment. They conclude that no definite therapeutic value can be attached to the use of Merthiolate in the treatment of pulmonary tuberculosis.

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Merthiolate in the Treatment of Tuberculosis. S. L. Cummins. *Lancet 233,* 962 (1937). Merthiolate killed tubercle bacilli, cultured on Besredka's fluid egg medium containing 1 per cent. Merthiolate, within twenty-four hours as revealed by subcultures. Intravenous injections of 2, 3, 4 and 5 cc. doses of a 1 per cent. solution of Merthiolate every second day for two weeks (the 5 cc. being repeated for each of the final four doses) were without beneficial effect in the treatment of fifteen tuberculosis patients. Irrigations in 1:1000 concentrations for a period of three weeks failed to clear up chronic tuberculosis sinus involvements.

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Vaccination of the Guinea Pig Against Tuberculosis with the Dead Bacilli Enrobed in Vaseline. A. Saenz. *Compt. rend. soc. biol. 125,* 495 (1937). Two groups of guinea pigs were treated with 10 mgm. of a heat-killed bovine strain of B. tuberculosis. Subcutaneous injections were given to the first group, the organisms being emulsified in physiological saline solution. In the second group the bacteria were coated in vaseline. The results of an extensive investigation indicate that coating dead bacilli with vaseline produces a vaccine which retards the dispersion of the organisms of superinfection but which does not confer immunity upon the animal so treated. Furthermore, there appears to be no parallel relationship between immunity and allergic reactions.

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An Experimental Study of Protective Inocculation with Heat-killed Tubercle Bacilli. Eugene L. Opie and Jules Freund.

J. Exptl. Med. 66, 761 (1937). Resistance against infection with virulent tubercle bacilli was increased by injecting heat-killed tubercle bacilli repeatedly into or below the skin of rabbits. The addition of some antigens especially heated horse serum, increased the protection given by heat-killed tubercle bacilli so that it is almost the same as that made possible by B. C. G. Heat-killed tubercle bacilli may be substituted for the living attenuated organisms so as to increase resistance against tuberculous infection and also to affect favorably the balance between asymptomatic or latent infection and progressive manifest disease so characteristic of human tuberculosis.

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Skin Reactivity of Men to Killed Tubercle Bacilli. E. Carlinfanti. *Policlicico 44,* 262 (1937), through *J. Am. Med. Assoc. 109,* 319 (1937). Different reactions were given by the same human subjects to Old Tuberculin injected into the skin and to an ointment made with tubercle bacilli killed at 158 degrees F. applied to the skin. The diagnostic and prognostic value of the ointment is regarded as more reliable than that of Old Tuberculin.

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Attempts at Vaccination Against Tuberculosis by Means of B. C. G. Vaccine Incorporated in Lanolin. I. Balteanu and A. Garaguli. *Compt. rend. soc. biol. 126,* 522 (1937). It has been demonstrated by others that the incorporation in lanolin of diphtheria and tetanus anatoxins greatly increases the antigenic properties of the latter. The results of this investigation reveal that incorporation in lanolin also increases the antigenic power of B. C. G. vaccine in experimental tuberculosis.

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Vaccination with B. C. G. R. Chaussinand. *Paris med. I, 15,* (1937), through *Am. J. Diseases Children 54,* 1125 (1937). Six hundred and seventeen infants were treated with B. C. G. employing different methods of administration. Most of these children observed through a period of several years never revealed a single pathologic incident which can be attributed to B. C. G. Communication from five obstetricians in Saigon who gave B. C. G. to 60,000 new-born

infants during the period of 1930 to 1936, revealed that these infants who remained under observation for from eight to fifteen days gave no gastro-intestinal disturbances which could be attributed to the ingestion of B. C. G. These clinical observations appear as valuable evidence of the innocuousness of B. C. G.

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Tissue and Humoral Reactions Following Absorption of B. C. G. by the Digestive Tract. Sol. R. Rosenthal. *Ann. inst. Pasteur 58,* 652 (1937). The oral administration of B. C. G. to guinea pig sucklings gave a general but not diffuse reaction of the reticulo-endothelial system. Animals inoculated with the organs of these guinea pigs were found at times to be sensitive to intradermal tests with dilute tuberculin. Cellular modification of the blood or peritoneal fluid which could be attributed to a tissue reaction toward B. C. G. was not found.

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Antituberculous Immunization of the Guinea Pig by Repeated Conjunctival Instillations with B. C. G. R. Schwarts. *Compt. rend. soc. biol. 127,* 205 (1938). Guinea pigs given twenty daily conjunctival instillations of 0.5 mgm. B. C. G. displayed resistance toward conjunctival infection, about two months after the beginning of immunization, by 1 mgm. virulent tubercle bacilli. The resistance conferred on guinea pigs by this method also extended toward subcutaneous infection with 0.001 mgm. virulent tubercle bacilli.

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B. C. G. Vaccination. K. Birkhaug. *Nord. med. tidskr. 1333* (Aug. 13, 1937), through *Brit. Med. J. II,* 94 (1937). Birkhaug who prepares B. C. G. for the Norwegian medical profession, states that B. C. G. cannot be accepted as a virus fixe. He also concludes that B. C. G. never produces progressive and fatal tuberculosis, not even in such susceptible laboratory animals as guinea pigs. He believes instead of anticipating that the present strains will increase in virulence in the course of time that actually evolution will occur in the opposite direction, and that B. C. G. will ultimately lose its

present ability to confer immunity to virulent tubercle bacilli. B. C. G. is the only available tubercle bacillus vaccine which stands out most prominently as an agent for increasing resistance to tuberculosis to a considerable degree. The intracutaneous method, which is highly recommended to the total exclusion of other methods, is more reliable than the oral method of administration. Social preventive measures which have proved effective for reducing the tuberculosis mortality are to be continued as a supplementary measure for tuberculin-negative persons exposed to infection from cases of open tuberculosis.

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Studies on Purified Protein Derivatives of Tuberculin (P. P. D.). Its Diagnostic Value and Keeping Qualities in Dilutions. Esmond R. Long and Florence B. Seibert. *Am. Rev. Tuberc. 35,* 281 (1937). Patients with clinical tuberculosis with but few exceptions react to the P. P. D. and most of them react to the smaller standard dose of 0.00002 mg. The exceptions are those with acute tuberculosis or serious chronic cases of long standing. Evidence is available that a part of the reactions to the second dose of P. P. D. is non-specific. Dilutions of P. P. D. lose strength on standing. The loss may be due partly to bacterial contamination which occurs even in the presence of antiseptic. It appears safe to keep the standard dilutions three days in the refrigerator.

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Summary of Results of Group Tuberculin Testing with P. P. D. (Purified Protein Derivative) in the United States: Final Report of the National Tuberculosis Association. Jessamine S. Whitney and Isabel McCaffrey. *Am. Rev. Tuberc. 35,* 597 (1937). A summary of a statistical study based on a total of 85,709 group tuberculin tests with first and second strength tuberculin (purified protein derivative) among 56,688 individuals in thirty states and the District of Columbia is presented. Among the 8276 persons reported to have had contact with tuberculosis, positive findings were obtained with 54.2 per cent. Only 33.3 per cent. of those with no history of contact responded with positive reactions. Of the 31,318 native born Americans of native parentage, 27.6 per cent. evidenced tuberculous infection, whereas 38.4 per cent. of the 6674 native born of foreign

stock and 61.2 per cent. of the 814 foreign born responded with positive reactions. Other interesting statistical observations are given.

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The Intracutaneous Quantitative Tuberculin Test in Active Pulmonary Tuberculosis. Joseph S. Pan. *Chinese Med. J. 51,* 979 (1937). Tuberculin tests on 406 persons gave 77.6 per cent. positives in active tuberculosis patients and 92.4 per cent. negatives in the normal control group. Most cases of advanced tuberculosis gave negative results. The ordinary tuberculin test appears to have little value in the differential diagnosis of active tuberculosis.

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Tuberculin Tests on 1054 College Students. M. W. Husband, C. M. Tice and D. T. Loy. *J. Kansas M. Soc. 38,* 420 (1937). Tuberculin tests on 1054 college students produced 336 (31.8 per cent.) positive reactors, 230 being found when using the first strength test and 106 with second strength test. Most unfavorable reactions (thirty) occurred with the use of the first strength test.

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Tuberculin Tests in Childhood. Edith M. Lincoln. *Preventive Med. 7,* 288 (1938). The technic and interpretation of the tuberculin test are considered. It appears that the Purified Protein Derivative of Tuberculin will replace Old Tuberculin, for diagnostic testing. The former, however, is more expensive and the stability of its solutions has been found to be low, so that the National Tuberculosis Association advises the use only of fresh solutions.

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Tuberculin Test in State 4-H Club Health Contestants. M. W. Husband and David T. Loy. *Diseases of the Chest 4,* 26 (1938). The one-test method of intradermal tuberculin testing with the intermediate dilution (0.0005 mg.) of P. P. D. is recommended.

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Technical Factors Affecting the Tuberculin Test. Waldo E. Nelson, Florence B. Seibert and Emmond R. Long. *J. Am. Med. Assoc. 108,* 2179 (1937). These workers caution against the use

of tuberculin syringes for any purpose where a tuberculin reaction might confuse the results. Positive Schick reactions have been obtained in Schick-negative individuals when the tests were made with syringes previously used for tuberculin. Similarly injections of physiological saline from such syringes after a simple washing and sterilizing give rise to positive reactions. Phenol in the usual concentrations does not cause a reaction simulating the tuberculin reaction. The most effective method of cleaning syringes to destroy all traces of tuberculin is boiling in soap solution and prolonged immersion in a sulfuric acid-dichromate solution.

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Further Studies with the Tuberculin Ointment Patch Test. Ernst, Wolff and Samuel Hurwitz. *J. Am. Med. Assoc. 109,* 2042 (1937). The agreement between the ointment patch test and the intracutaneous test with 0.1 mgm. of Old Tuberculin in 1075 observations was 98.2 per cent. Discrepancies occurred only in clinically latent cases. The ointment test was positive in every case of active tuberculous disease. The results warrant the conclusion that the patch test may safely be substituted for the Mantoux test.

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The Detection of a Biological Difference Between Old Tuberculin and Bovine Tuberculin by the Schultz-Dale Technique. P. W. Schmidt and B. Bausch. *Klin. Wochschr. 17,* 744 (1938). The Schultz-Dale technique reveals that Old Tuberculin and Bovine Tuberculin contain a common antigen and also type specific antigens.

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A Comparison of Intracutaneous Reactions in Man to the Purified Protein. Janet McCarter, H. R. Getz and R. H. Stiehm. *Am. J. Med. Sci. 195,* 479 (1938). The tuberculin test is of value in a public health program for the detection of tuberculosis in a large group, as it can be used to select those who should have chest X-rays. It should be used however as an indication rather than as an exact measure of the extent of infection with tubercle bacilli in the general population.

Uveal Tuberculosis. George O. Meyer. *J. Med. Soc. New Jersey 35,* 138 (1938). In uveal tuberculosis, specific desensitization with tuberculin is the most important curative measure. Subcutaneous injections are advocated with the initial dose being one-tenth of the minimum dose of Old Tuberculin (or its equivalent in other products) sufficient to elicit a positive skin reaction. The increase depends upon the presence of local and general reactions. A maximum of 25 or 50 mgm. per dose may be given.

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A Diluent for Stabilizing Tuberculin "O. T." Diluted for the Mantoux Test. Russell, Gottschall and Wm. E. Bunney. *J. Immunol. 34,* 103 (1938). A diluent buffered to pH 7.2 with boric acid and borax, containing 0.04 per cent. gum arabic and 0.5 per cent. phenol was found to stabilize tuberculins tested when diluted 1:10,000 and dispensed in rubber stoppered hard glass vials of 1 or 10 cc. capacity. Shaking for seven days, exposure to indirect sunlight at room temperature for four months, or prolonged transportation to warm climates and return did not affect the stability.

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Development of Local Cellular Reaction to Tuberculin in Sensitized Calves. William H. Feldman and C. P. Fitch. *Arch. Path. 24,* 599 (1937). The local reactions following the injection of a diagnostic dose of mammalian tuberculin into the derma of each caudal fold of nine different calves which had been infected with bovine tubercle bacilli and two control calves which had not been infected, was studied by biopsy at three, six, twelve, eighteen, twenty-four, thirty, thirty-six, forty-two, forty-eight, fifty-four, sixty and seventy-two hours and five, seven, ten, fourteen, twenty-one and twenty-eight days after the injections. Evidences of a constant predilection for the perivascular and perineural tissues were noted. Polymorphonuclear leucocytes were numerous during the early phase of the reaction which were gradually replaced by a histiocytic or mononuclear cellular reaction which predominated at the end of sixty to seventy-two hours. Edema appeared early in the reaction and disappeared between the fifth and seventh day. Resolution of the cellular reaction had not occurred after twenty-eight days. Endo-

vascular changes, including thrombosis and endarteritis occurred. The injection of tuberculin into the skin of non-sensitized calves did not give demonstrable changes.

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The Nature of the Tuberculous Antibody of Human Serum. Kurt, Meyer. *Compt. rend. soc. biol. 127,* 947 (1938). In 145 serums from human cases of osteo-articular tuberculosis some gave reactions with the lipid antigen from the tubercle bacilli, others with the polysaccharide antigen and in some cases the reactions were with both antigens. The presence of lip-polysaccharide antibody was found in some of the sera.

Third Skin

This preparation, *which is patented* and which would seem to fill a long-felt need for many industrial workers, is claimed to protect the hands against dirt, grease, paint, oil, ink, gasoline, acids, alkalis, and so on.

This mixture is said to be invisible, elastic, permits the passage of perspiration without loss of its protective properties and to persist in this property for at least eight hours. It may be removed by washing with water. This "third skin" consists of:

Sodium soap	128 oz.
Water glass	110 oz.
Glycerine	100 oz.
Potato starch	100 oz.
Distilled water	2 oz.
Cottonseed oil	32 lbs.

Perfume, if desired, a few drops.

Such a glycerine-containing preparation may be expected to be especially useful for painters, printers, gasoline-station attendants, automobile mechanics, and in short, all workers who come in daily contact with clinging substances that are difficult to remove from the skin. The use of the coating is not limited to the hands alone, but may be used on any part of the skin where protection is desired. The inventor designates his invention as the "third skin" because it covers the two natural skins of the body.

(N. B. The patent referred to is U. S. Pat. No. 2,120,569, Osmer F. Oliver, of Akron, Ohio.)

SOLID EXTRACTS

By Ivor Griffith, Ph. M., Sc. D.

Despite the form in which this information is presented it may be accepted as trustworthy and up-to-date. Original sources are not listed but they may be obtained upon request.

When new books annoy, the Bible affords a fine solace. And although the family Bible is no longer the *edifice,* the *institution* it once was, there are still those who find within its pages more, much more, than may be found within the pages of any other book—more of comfort, more of peace and indeed more of a pulsing poignant history than the casual reader ever imagines. Of course there *must* be a reading between the lines.

For instance, to persons interested in medical history what other than diabetes might be the diagnosis of the disease of which Asa the King of Judah died. Diseased was he in the feet, more than likely a diabetic gangrene, "Yet in his disease he sought not to the Lord, but to the physicians" (II Chronicles xvi, 12). Within two years the king had died of this disease. And his people "laid him in the bed which was filled with sweet odours and divers kinds of spices prepared by the apothecaries art."

In all the Bible there is very little reference to any internal medicine but only to ointments and perfumes and spices. Medicines and the apothecary are mentioned many times, however, in the apocryphal book of Ecclesiasticus. Among much mention of medicines in Jewish lore of the times is an alleged specific for King David's spells of melancholia, in the form of an after-dinner pill of aloes and myrrh, saffron, opium and spices, flavored and rolled with honey.

Indeed the word *melancholia* itself is diagnostic, for it suggests a gall-bladder involvement.

"And when Youth the dream departs,
It takes something from our hearts
And it never comes again."

So sang a poet of yore. But the experimenting physiologists laugh at the sentiment expressed and continue the quest of the

alchemist for that elusive elixir which may some day bring to mankind the boon of a more protracted happy period of youthfulness.

Old men can be made young again, mentally as well as otherwise, by means of hormone injection, Dr. Neal E. Miller, of the Institute of Human Relations, Yale University, recently told American Psychological Association.

Elation takes the place of depression in most of the patients, Dr. Miller observed in the course of an experiment in which the effect of injection of the hormone testosterone proprionate was compared with results of a similar injection not containing the hormone. The group included, in addition to the cases of old men being rejuvenated, a number who were suffering from various types of glandular deficiency. Improvement was greatest when the deficiency had been greatest. Rational aggressiveness took the place of irrational irritability, for some patients. Nervousness and emotional instability were decreased. Muscle tonus, energy and stamina returned.

As Galileo observed—"The world does move!"

Morphine is still considered one of Nature's greatest medicinal gifts to man, a priceless boon in pain appeasement. Yet its double-dealing, habit-forming property has forever been its crime and curse.

But now comes a new pain-relieving drug which may be the means of freeing the world from the poppy's bondage.

The drug, dinitrophenylmorphine, was first reported by Dr. Chauncey D. Leake, professor of pharmacology at the university, at the meeting of the British Pharmacological Society at Oxford University.

The new drug, called DNPM for short, is a combination of morphine and dinitrophenol. The latter is a fever-producing drug which caused disastrous results and some deaths when used without proper supervision as a weight-reducing medicine. The new drug is said to have none of the action of dinitrophenol but to be much more like codeine and morphine.

Experiments on animals and normal human subjects show that it has pain-relieving properties and respiratory effects similar to morphine and greater than codeine. Animal experiments also suggest that it may be less habit forming than morphine.

A summary of the Einstein theory insists that we do not know where we are, or where we are going unless we know what time it is. The watch affords us time of day—and the compass direction. The compass, by the way, judged by most to be a modern invention, is older than we know. Thus William the Clerk, a monk, describes a way to make one from very primitive elements:

"Who would of his course be sure,
When the clouds the sky obscure,
He an iron needle must
In the cork wood firmly thrust.
Lest the iron virtue lack
Rub it with the lodestone black,
In the cup with flowing brim,
Let the cork on water swim.
When at length the tremor ends,
Note the way the needle tends;
Though its place no eye can see—
There the polar star will be."

Omar, the tent maker, knew more intimately of chemistry than did the so-called scientists of his day. His quatrains explain more clearly the nitrogen cycle than do many of the diagrams in our text-books. For instance:

He states in terser terms exactly what the following paragraphs taken from a modern lecture are meant to convey.

Let Life desert this fragile human temple, rot and decay soon start their decomposing work. The germs of putrefaction are no respecters of persons and a kingly shroud deters their wrecking enterprises no more than the beggar's coverlet. The silent dissolution of the material flesh proceeds with equal regularity and relentlessness in Potter's field and in the marble vaults of Croesus.

Nature demands again the ingredients that she loans to the soul's repository, and she is more anxious about her fixed nitrogen than any part of the carcass. Of course every atom of the body is put to another use. Let none be misled into thinking that death is the end of even material things.

"There is no death.
The dust we tread
Shall change beneath the summer showers
To golden grain or mellowed fruit,
Or rainbow-tinted flowers."

So often the real pioneers fail to live long enough to gain the benefits of their dreams and deeds. Thus died Moses short of the promised land.

In the field of chemical pioneering this is particularly true. Mercer never profited from his discovery of mercerization. Leblanc, discoverer of that famous method for making "soda" from salt, that is, sodium carbonate from sodium chloride, died a pauper. His early production of soda ash on a commercial scale in a factory erected at St. Denis, near Paris, under the support of the Duke of Orleans, was interrupted by the dark days of the French Revolution. The Duke of Orleans was guillotined, the factory confiscated and Leblanc forced to reveal his process to the State. Though his factory was returned in 1800, Leblanc was unable to secure financial help and in 1806, in poverty and despair, he took his own life. In 1824 James Muspratt, in England, undertook the commercial production of soda ash by the Leblanc process and with great success, the process proving to be of untold benefit to the world, especially as soda ash is also an essential ingredient in glass manufacture.

BOOK REVIEWS

Done by persons, unafraid to upbraid, but perfectly willing to give praise where praise is really due.

A College Textbook of Pharmaceutical Botany. By Heber W. Youngken. Sixth Edition, 793 pages. P. Blakiston's Son and Co., Inc. Philadelphia, 1938.

Teachers of botany in general and of pharmaceutical botany in particular have been delighted recently by the appearance of the sixth edition of Dr. Youngken's well-known work. Stimulated by the appearance of the U. S. P. XI and N. F. VI, and at the same time with a full realization of the needs of the student in the general course in college botany, Dr. Youngken has prepared a revision which not only treats his subject from the broad academic viewpoint, but which is also particularly and peculiarly adapted to the needs of the student of pharmacy. His aims are cultural as well as professional.

Many changes have been made to improve the text, such as the introduction of chapters on "The Living Cell," "Non-protoplasmic Cell Contents" and "Genetics and Evolution"; the amplification of the material dealing with plant tissues and plant organs; the enlargement of the "Glossary" and "Classified List of Reference Works."

The chapters on the "Microscope" and "Histological Technique" have been given the status of Appendix I and Appendix II respectively. In the latter, the use of N-Butyl alcohol in imbedding woody plant organs is described.

There are 121 added figures, making a total of 507.

The type is large and easy to read, and the volume is attractively and durably bound.

There is no need to remind readers of Dr. Youngken's other editions that he possesses to an unusual degree the ability of clear and concise description.

Altogether, the sixth edition is a volume which should be on the "must list" of those interested in botanical science, whether teacher or student.

M. S. DUNN.